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## Transmission dynamics of a trematode parasite: exposure, acquired resistance and parasite aggregation

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**Abstract** This paper examines the relative importance of exposure and susceptibility to the infection of rainbow trout (*Oncorhynchus mykiss*) with the trematode parasite *Diplostomum spathaceum* under natural conditions. A total of 93 individually marked, similarly aged fish were introduced into three cages at regular time intervals and the intensity of infection in individuals recorded by counting parasites in live fish using ophthalmic techniques. Fish introduced into the cages became infected faster than fish that were already in the cages, indicating that fish developed resistance to infection after repeated exposure. Fish kept in the cages experienced similar levels of exposure and the distribution of parasites between these fish was not significantly different from a random distribution. In contrast, parasites from 16 Finnish wild roach populations were highly aggregated. The differences between the caged fish and the wild fish indicate that the aggregated distribution in wild fish might be determined by variations in exposure rather than variations in susceptibility between fish. This is one of the few studies to demonstrate the development of resistance in fish against the parasite under natural conditions, and to attempt to separate exposure and susceptibility as causative agents of parasite aggregation.

### Introduction

An understanding of parasite transmission requires not only an estimate of the rate at which hosts contact infective stages but also an estimate of how the host acquires resistance against further infection (Hudson et al. 2002). However, obtaining an estimate of acquired resistance against macroparasite infections in wild animal hosts suffers from two methodological limitations. First, direct counts of the intensity of infection are usually not possible without either the death of the host or the application of an indirect method that incorporates further measurement errors (Wilson et al. 2002). Secondly, since workers wish to estimate susceptibility they usually standardise exposure experimentally using an artificial infection with pulsed exposure, which is unnatural and usually does not correspond to either the natural level or the pattern of exposure (for review, see Hellriegel 2001). Indeed, in many instances the challenge exceeds natural infection rate by many orders of magnitude.

To study the development of acquired resistance we ideally require a longitudinal study of wild living individuals exposed to natural levels of infection where we can record the intensity of infection in marked individuals. One system that permits the monitoring of natural levels of infection within the same individuals is the infection of salmonid fish with the eye fluke *Diplostomum spathaceum*. Using ophthalmic techniques (see Wall and Bjerkås 1999), we have developed a method for repeatedly counting the number of established worms in the eyes of individually marked fish and recording changes in the intensity of infection over time. By placing individually marked, equal-aged fish in restricted cages in a natural lake system and adding tracer fish at time intervals, we can record both the natural rates of infection and the development of acquired resistance by comparing these in temporally overlapping, consecutive fish cohorts.

Previous workers have presented age-intensity data from caught fish and provided evidence that the number

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of new *Diplostomum* spp. infections in fish decreased with age so that the age-intensity curve reached an asymptote (e.g. Wootton 1974; Burrough 1978). One possible mechanism for this pattern is the development of resistance. However, age-intensity data cannot be used to confidently describe the development of resistance since these data will be confounded by age-related variations in exposure. These factors need to be controlled experimentally if we are to obtain a clear insight into the development of acquired resistance. Nevertheless, laboratory studies have examined the physiology associated with resistance against *D. spathaceum* and identified the presence of both specific and non-specific immune responses to infection (Bortz et al. 1984; Stables and Chappell 1986; Whyte et al. 1987, 1989, 1990; Höglund and Thuvander 1990). Specific antibodies have been detected 6 weeks after infection (Whyte et al. 1987), although the immune response can be dependent on the ambient water temperature (Ellis 1988; Manning and Nakanishi 1996). Furthermore, Speed and Pauley (1985) have examined the response of fish to different amounts of antigen. In nature, exposure to parasites varies between individual hosts because of variation in host behaviour and the aggregated distribution of the infective stages within the fish habitat. Variations in exposure coupled with variations in the response of the host may lead to an aggregated distribution of parasites within the fish population.

Most parasites are highly aggregated within their host population, with the majority of parasites established in a relatively small proportion of the host population (Shaw and Dobson 1995; Shaw et al. 1998; Wilson et al. 2002). The aggregated distribution of *D. spathaceum* in its fish hosts has been reported on several occasions (e.g. Pennycuik 1971; Sweeting 1974; Burrough 1978). A fundamental question in epidemiology has been to ask what are the important factors that influence this pattern of distribution? Is this a consequence of variations in susceptibility or exposure between individuals, or a consequence of the sampling methods?

The aim of the present paper was to study the relative importance of susceptibility and exposure in generating the pattern of *D. spathaceum* distribution within fish populations. We approach this issue by addressing two specific questions: (1) Do host fish develop resistance to natural levels of infection? (2) How does the degree of parasite aggregation in fish with similar patterns of exposure compare with natural populations with presumably variable exposure?

## Materials and methods

### *Diplostomum spathaceum*

The taxonomy of the genus *Diplostomum* spp. is problematic and not completely resolved (see Valtonen and Gibson 1997). Fish in northern Finland carry two lens forms (Valtonen and Gibson 1997), and the majority of the specimens resemble *D. indistictum*, referred to as *D. spathaceum* by Niewiadomska (1986). In this

study, we therefore considered parasites located in the lens as *D. spathaceum*, but recognise that other species might also be present. The life cycle of the parasite includes three hosts, beginning with the definitive host, the fish-eating bird, where sexual reproduction takes place. Cercariae are formed through asexual reproduction within the first intermediate host, the freshwater snail *Lymnaea* spp., and are released into water to infect several species of second intermediate fish hosts. Once cercariae come in contact with fish, they migrate through fish tissues and settle in the lens of the eye, where they form a long-lived metacercarial stage (Chappell et al. 1994).

### Experimental set-up

The experiment was conducted in three cages in Lake Konnevesi in Central Finland between 30 June and 4 September 2000, a period of 66 days. One-year old rainbow trout [*Oncorhynchus mykiss*; mean length and weight  $\pm$  SE:  $193.9 \pm 2.15$  mm and  $82.7 \pm 2.94$  g, respectively] were obtained from a commercial fish farm where they had acquired a low level of infection. One month prior to the experiment, each fish was studied for *D. spathaceum* infection under anaesthesia (MS-222 as anaesthetic) using a slit-lamp microscope (see Wall and Bjerkås 1999 for methodological details) and received an individual tag (soft VI Alpha Tag) for subsequent identification. All fish were maintained in indoor tanks prior to the experiment and fed with commercial fish pellets. Each cage was 120×80×100 cm, made of wood strips and covered with soft net (mesh size 10 mm), which allowed the penetration of cercariae. Cages were considered large enough not to restrict the fish but small enough to provide equal conditions and exposure within the cage. Cages were placed in shallow lake water (depth ca. 1.5 m) amongst the densely vegetated littoral zone where the intermediate snail hosts for the parasite were known to reside.

The experiment was designed as a tracer experiment where each cage would contain fish from three overlapping fish cohorts introduced at different times during the experiment, taking account of natural variations in exposure. The experiment started on 30 June when ten randomly chosen fish (referred to as group I) were introduced into each of the three cages. Subsequent fish were added to all cages on day 24 of the experiment (referred to as group II), when the total number of fish in cages was adjusted to 20. The third group of fish was added on day 46, when the total number was adjusted to 15. The total number was lower because of mortality of fish in the previous groups (see below). Water temperature was between 16.1°C and 20.7°C during the experiment. Fish were fed every second day with commercial fish pellets, and the average increase in fish length and weight per day  $\pm$  SE was  $0.88 \pm 0.08$  mm and  $1.84 \pm 0.18$  g, respectively, indicating that the fish were growing normally during the experiment. All fish from the cages were examined for *D. spathaceum* infection under anaesthesia using the slit-lamp microscope on days 13, 24, 35, 46 and 56 of the experimental period. The experiment was terminated on day 66 when all fish were dissected. The initial objective of the study was to follow fish groups I–III until the end of the experiment. However, due to the relatively high mortality of fish in group I, these fish were dissected on day 46. The mortality (fish group I: 16 of 30 fish, fish group II: 21 of 41 fish, fish group III: no mortality) occurred mainly in July and was probably associated with high water temperatures during that time.

While ophthalmic techniques allow us to obtain a reasonable estimate of intensity they may under-estimate abundance when worms lie behind each other in the eye. On the other hand, counts may over-estimate abundance when metabolic excretions of the parasites, or sometimes lens abnormalities, are misinterpreted as parasites. Other workers (e.g. Chappell et al. 1994) believe that *D. spathaceum* metacercariae do not die while in the lens, at least not in the time scale used in the present experiment, so we considered the count taken at post mortem dissection to be a correct estimate of intensity at the end of the experiment.

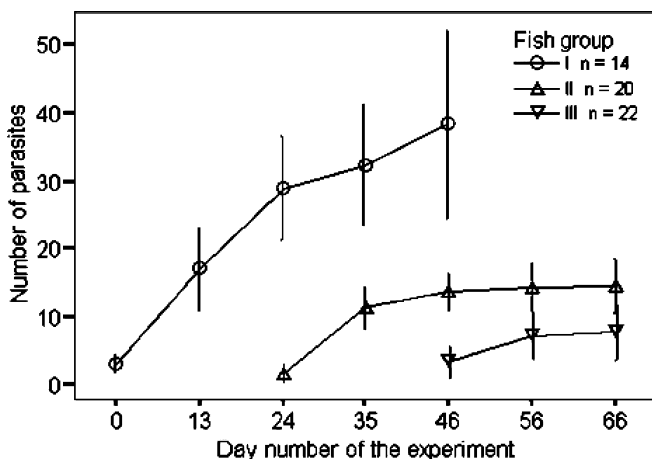
## Parasite aggregation

The degree of *D. spathaceum* aggregation in experimental rainbow trout was compared to that in 16 wild roach (*Rutilus rutilus*) populations in Finland (Valtonen and Gibson 1997; Valtonen et al. 1997; our unpublished data; J. Taskinen unpublished data). Naturally infected rainbow trout could not be used for the comparison since wild populations do not occur in Finland. Furthermore, roach frequently harbour *D. spathaceum* metacercariae of comparable intensity to those recorded in our experimental fish, so we compared data between species. The relationship between log mean and log variance in parasite numbers was then used to interpret the degree of parasite aggregation in both the experimental fish and the roach (see Taylor 1961, 1970).

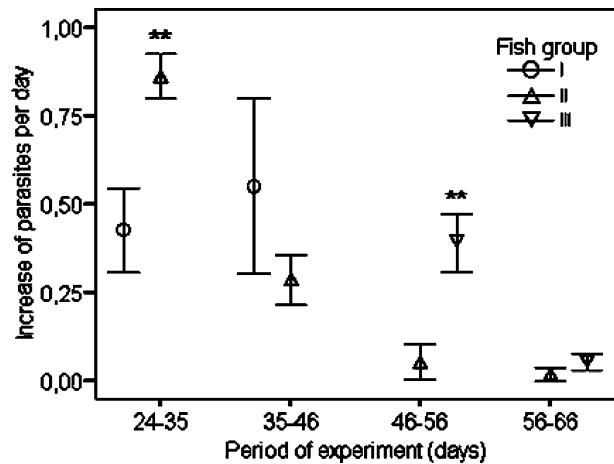
## Results

The intensity of parasite infection increased with the time period the fish were held in the cages in all fish cohorts (Fig. 1). The infection did not differ between cages (ANOVA:  $F_{8, 47}=0.48$ ,  $P>0.7$ ) and therefore data from the cages were pooled. The increase in parasite numbers was most pronounced in fish in the first group, with lower rates of infection in subsequent groups (ANOVA:  $F_2=117.91$ ,  $P<0.001$ ). The initial infection intensity, at the start of the experiment, did not differ between the three fish cohorts (ANOVA:  $F_2=2.00$ ,  $P>0.1$ ). The parasite intensity in fish that died during the experiment was not significantly different from those that survived (ANOVA:  $F_1=2.66$ ,  $P>0.1$ ) and therefore all subsequent analyses were performed using data from fish that survived throughout the experimental period.

In the analysis, the increase in parasite numbers per unit time was first compared between temporally overlapping fish groups I and II, first during days 24–35, and then during days 35–46 of the experiment (see Figs. 1, 2). The fish density in cages was not constant during the whole experimental period because of introduction of new fish groups and mortality of fish. However, the density was maintained constant during each period



**Fig. 1** The mean number of *Diplostomum spathaceum* parasites in three temporally overlapping fish cohorts held in three cages in a lake system (fish that survived the experiment, all three cages combined) and exposed to natural infection. Bars  $\pm 1$  SD

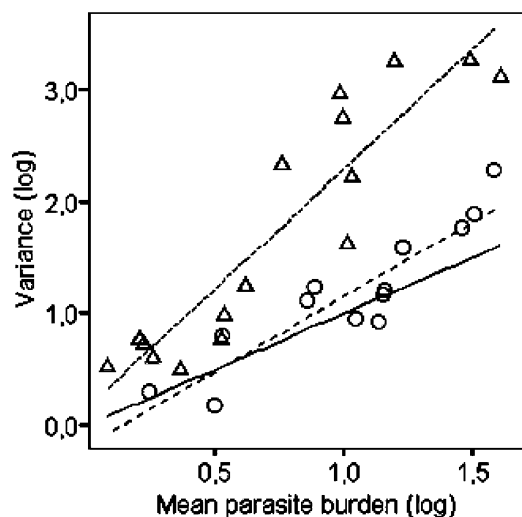


**Fig. 2** Increase in the numbers of *D. spathaceum* parasites per day in three groups of individually marked fish held in three cages in a lake system. \*\* Statistically significant differences in parasite accumulation within a period ( $P<0.01$ , see text). Bars  $\pm 1$  SE

compared, which excluded the effect of changing fish density on the rate of parasite acquisition. There was a significant interaction between the increase in parasite numbers and the classifying variable, fish group (repeated measures ANOVA:  $F_1=6.561$ ,  $P<0.05$ ; Fig. 2), and therefore the simple effects were analysed using *t*-test. The analysis showed that fish in the second group became infected with more parasites than the first group during the same period of exposure (days 24–35; *t*-test:  $t_{31}=-3.428$ ,  $P<0.01$ ), but these differences were not observed in the following period (days 35–46; *t*-test:  $t_{31}=1.164$ ,  $P>0.2$ ; Fig. 2). Similarly, a significant interaction was also found between the fish groups II and III during the days 46–56 and 56–66 of the experiment (repeated measures ANOVA:  $F_1=10.885$ ,  $P<0.01$ ; Fig. 2). Again, parasite numbers in fish group III increased faster after introduction in comparison with fish group II (days 46–56; *t*-test:  $t_{40}=-3.313$ ,  $P<0.01$ ), but no difference was observed in the following period (days 56–66; *t*-test:  $t_{40}=1.168$ ,  $P>0.2$ ; Fig. 2).

In the last period of the experiment (days 56–66), in just one of the cages, parasite numbers again increased significantly (ANOVA:  $F_1=38.65$ ,  $P<0.001$ ). This increase was probably due to high output of cercariae by nearby snails as dissection of these fish revealed large amount of newly established parasites, distinguished according to their smaller size (see Sweeting 1974). Since this took place only in one of the cages, we considered this an accidental event and excluded the number of small parasites from these fish when analysing the infection data, but we discuss the result further below.

The relationship between log variance and log mean of sampled populations has been used by Taylor (1961, 1970) and others (e.g. Valtonen and Niinimaa 1983; Shaw and Dobson 1995; Poulin and Morand 2000; Boag et al. 2001) as a means of examining the characteristic pattern of parasite aggregation within a host population.



**Fig. 3** The relationship between log variance and log mean in the numbers of *D. spathaceum* parasites in the experimental fish (circles) and in 16 wild roach populations (triangles). The solid line represents the 1:1 relationship between log variance and log mean above which the parasites are aggregated

In the experimental fish system, the log variance increased with respect to the log mean at a rate not significantly different from unity [slope =  $1.34 \pm 0.18$  (SE), 95% confidence interval = 0.980–1.702], indicating that the parasites were not aggregated within their host population (Fig. 3). Note that the observations from the experimental fish in Fig. 3 are not independent since they represent repeated measurements of parasite burden from the same fish individuals at different time points. This was done because we were interested in the pattern of aggregation and is unlikely to have any influence on the conclusions drawn. In contrast to experimental fish, the log variance in roach populations increased faster than the mean [slope =  $2.15 \pm 0.25$  (SE), 95% confidence interval = 1.656–2.636] showing the parasites were aggregated within these fish (Fig. 3).

## Discussion

This paper explored the development of resistance in fish hosts against the macroparasite, *Diplostomum spathaceum*, in a lake system under natural infection conditions. We concentrated on studying resistance in an ecological setting by repeatedly recording parasite intensity from the study fish and did not consider processes or mechanisms of immunity. Analysis of the data showed that newly introduced fish became infected with significantly more parasites than fish that had already been exposed, indicating that the latter fish had developed resistance against the parasite as a result of previous infections. This assumes that the infection did not change the behaviour of fish during the experiment so that it would have altered exposure, which seems reasonable considering the small infection

scale used in this study (see below). It was also assumed that intraspecific parasite competition does not occur, or is insignificant, in the lens. This also seems reasonable given the relatively low intensities observed in the study fish when compared to other studies (Wootten 1974; Field and Irwin 1994; Marcogliese et al. 2001). The acquisition of parasites also decreased with the season, which is consistent with the findings of previous workers (e.g. Wootten 1974; Burrough 1978; McKeown and Irwin 1997), and is most likely a consequence of decreasing output of cercariae from snails. We assumed that all cercariae released from snails during the experiment were of similar quality, which is supported by our previous results (Karvonen et al. 2003). Furthermore, the water temperature was relatively constant during the experiment (16.1–20.7°C) and therefore it is unlikely to have affected the quality or activity of cercariae.

The overall pattern of parasite aggregation was not significantly different from a random Poisson distribution in the experimental fish, but wild roach exhibited a highly aggregated distribution. These comparisons lead us to suppose that, in this system, the aggregated distribution observed in the wild is probably not a consequence of variations in susceptibility but a consequence of variations in exposure between individuals. Of course this assumes that the fish in our experiment were exposed to similar numbers of cercariae during the period of the experiment, which seems reasonable since the fish were kept in relatively small cages. Furthermore, the results imply that individual variations in susceptibility might not be important in generating the aggregated distribution. However, we must note that we made comparisons with wild roach, a native fish species with probably greater genetic variability than the captive bred rainbow trout we used in our experiment. However, maintaining wider genetic variability in fish through frequent cross-fertilisation between different stocks is a common practice in fish farming, which causes individual differences in susceptibility between the trout.

Furthermore, this experiment was started with fish that were not totally naïve to infection, as all individuals harboured a low initial infection, but we suggest that this infection probably had no effect on the final results because the infection intensity was low and did not vary between fish groups. The most likely consequence from this infection would be a decrease in subsequent infection if some degree of initial immunity had developed, but this could not be analysed from the present data. Secondly, we applied a slit-lamp microscope for the first time in fish parasitology to repeatedly record the parasite burden in fish. We recognise that this method may be subject to error when parasite numbers are high. However, the parasite numbers shown by post mortem dissection at the end of the experiment were close to those recorded using the slit-lamp microscope [mean % difference =  $8.1 \pm 2.3$  (SE)], which implied that this method captured the pattern of infection with the infection intensities in this experiment.

To our knowledge this is one of only a few studies to demonstrate the development of acquired resistance in fish against *D. spathaceum* under natural conditions using an experimental approach that separated the effect of resistance from changes in exposure. A number of workers have described the development of immunity physiologically against the parasite under laboratory conditions (e.g. Bortz et al. 1984; Stables and Chappell 1986; Whyte et al. 1990; Höglund and Thuvander 1990). However, natural infection conditions usually provide continuous, variable, moderate level exposure, in contrast to the single high-level infection used in experiments, and thus may provide more realistic insight into development of resistance. This pattern of infection may affect the mechanisms determining how development of resistance actually takes place, which again may affect the ability of resistance to protect the host in subsequent exposure and against the deleterious effects of infection within the host (see below). Furthermore, all studies conducted in the laboratory (see references above) have also invariably used rainbow trout as a model species, presumably because of the commercial value and easy availability. However, to obtain a good insight into population dynamics and transmission of the parasite in nature, we would ideally need immunological data also from wild, native fish species (see Aaltonen et al. 1997). Such data, which consider the role of resistance or immunology in transmission dynamics of fish macro-parasites, are scarce (but see Lysne and Skorping 2002), but needed from various parasite-host systems.

In addition to studying the development of resistance itself, an important factor in ecological immunology is the ability of acquired resistance to protect the host during subsequent infection in such a way that the harmful effects of the parasite can be avoided. Our data indicate that the parasite might overcome this protection to some extent as parasite numbers in fish in one of the cages increased at the end of the experiment following an increase in exposure. Heavy infections in fish by *D. spathaceum* may induce the development of cataract (e.g. Shariff et al. 1980), which may reduce growth and feeding efficiency of the infected fish (Crowden and Broom 1980; Owen et al. 1993; Buchmann and Uldal 1994) and may thus have deleterious effects on the host. However, our present data do not show if the observed increase in parasite numbers would have actually induced the harmful effects in fish at a later stage despite the acquisition of resistance, and therefore the efficiency of resistance could not be analysed. Infections in nature are characterised by spatial and temporal variation in exposure between host individuals, which impose different degrees of pressure on defence systems and ultimately may determine how effectively these systems can protect the host. We therefore need experimental set-ups with natural infection conditions to investigate the efficiency of resistance in protecting the fish against the parasite and against cataract formation; this topic forms the basis for a subsequent paper.

Our studies in this particular parasite-host system imply that parasite aggregation may rise through differences in exposure between hosts since the parasites were not aggregated in the presumably evenly exposed caged fish compared to wild fish. The relative importance of exposure and susceptibility in generating aggregated parasite distributions is of wide interest in population and evolutionary dynamics of parasite-host relationships (Wilson et al. 2002). Theoretical models predict that even small variations in susceptibility between hosts may rapidly produce aggregated parasite distribution in the host population (Anderson and May 1978). However, empirical data effectively separating these factors are scarce. One example is the study by Tanguay and Scott (1992), who found that heterogeneity in acquired resistance between individual hosts was mainly responsible for the observed parasite aggregation in a nematode-mouse system. Furthermore, Lysne and Skorping (2002) found that infection rates of a parasitic copepod varied between individual fish because of differences in susceptibility. Parasite aggregation in wildlife hosts probably results from complex interactions between aspects in host behaviour, acquired and innate resistance, and genetic factors, the relative importance of which is likely to vary between different host-parasite systems. We therefore emphasise that these few studies require continuation in varying parasite-host systems if we aspire to find general trends in the causes of aggregation, for example between related parasite or host species.

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